Role of complement in the pathogenesis of postpartum thyroiditis: relationship between complement activation and disease presentation and progression

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The aim of this study was to assess whether the presentation and progression of autoimmune postpartum thyroiditis (PPT) was related to the degree of thyroid peroxidase autoantibody (TPO-ab)-mediated activation of the complement cascade. One hundred and forty-eight thyroid autoantibody-positive women have been followed during their postpartum year. Seventy-five women remained euthyroid during this time whilst the remaining 73 showed one or more episodes of thyroid dysfunction. Fourteen women showed hyperthyroid PPT, 23 showed a biphasic PPT and the remaining 36 showed hypothyroid PPT. Hyperthyroid PPT always transient but 29 of the 59 women with hypothyroidism remained hypothyroid or still required thyroxine replacement therapy at the conclusion of the study. Thyroid autoantibodies were measured by enzyme-linked immunosorbent assay (ELISA), free triiodothyronine and free thyroxine by the Amerlex M methods and thyrotrophin by the Amerlite TSH (monoclonal) assay. Complement component C3b, immobilized as a result of classical complement pathway activation in the presence of TPO/TPO-ab complexes in vitro, was measured by ELISA. Bioactive TPO-ab were calculated as the product of the C3 index and TPO-ab level. Basal levels of complement C3 activation were seen in the euthyroid TPO-ab-positive women (C3 index 0.06 at delivery rising to 0.36 at 12 months postpartum; bioactive TPO-ab activity 0.4 kIU/l at delivery rising to 10.4 kIU/l at 12 months’ postpartum (N = 75). These parameters were elevated progressively as the severity of the clinical syndrome increased. In 14 hyperthyroid PPT women the C3 index was 0.47 at 8 months’ postpartum (bioactive TPO-ab activity = 20 kIU/l; p vs euthyroid group, NS). In 23 biphasic PPT women the C3 index had risen to 0.65 by 7 months’ postpartum (p < 0.005 vs euthyroid group), with bioactive TPO-ab activity 81 kIU/l (p < 0.005), and in 36 women with hypothyroid PPT the C3 index was 0.7 by 6 months’ postpartum (p < 0.005), with bioactive TPO-ab activity 76 kIU/l (p < 0.005). In women with persistent PPT the C3 index had risen to 0.76 by 5 months’ postpartum, with bioactive TPO-ab activity 116 kIU/l; both parameters were statistically higher in the persistent group than in the remaining 44 cases where the C3 index was 0.56, with bioactive TPO-ab activity 33 kIU/l (p < 0.005 vs euthyroid; p < 0.05 vs transient). This study of the role of complement in the pathogenesis of PPT shows that the severity and duration of the thyroid dysfunction correlates with the degree of complement activation by TPO-ab measured in vitro.

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Postpartum thyroiditis (PPT), an autoimmune syndrome not unlike Hashimoto’s thyroiditis (1), affects 3.7–5.9% of women during the first year postpartum (2). It is a destructive thyroiditis characterized by a high rate of iodine excretion (3) and elevated serum thyroglobulin levels (4) at 3 months’ postpartum and is strongly associated with the presence of elevated levels of circulating thyroid peroxidase autoantibody (TPO-ab), the microsomal antibody seen in 10% of women at booking (approximately 16 weeks of pregnancy) (5–10). This condition presents with an initial episode of hyperthyroidism followed by hypothyroidism or separate episodes of either, and is reported to lead to permanent hypothyroidism in some 30% of cases (11–13).

Recently (14), we described the involvement of the complement system in the pathogenesis of PPT, showing that complement activation by TPO/TPO-ab complexes in vitro was higher in women with PPT compared with that seen in a group of euthyroid TPO-ab-positive control women. This paper shows that the degree of TPO-ab-mediated complement activation by serum samples from women with PPT is related to the

*Deceased
severity of thyroid damage, as indicated by the type and duration of their thyroid dysfunction.

Methods

Patients
The patients described in this paper took part in a study of the incidence, prevalence and clinical consequences of postpartum thyroiditis in a South Wales community carried out between 1987 and 1989 (15). Ethical approval for the study was obtained from the Mid Glamorgan Health Authority, South Wales and all participants gave their written informed consent. A consecutive series of 1996 women who presented at about 16 weeks’ gestation to the booking clinic of the Caerphilly District Miners Hospital, Mid Glamorgan, UK were screened for the presence of circulating thyroid autoantibodies. One hundred and fifty-two of the 235 antibody-positive women together with 239 antibody-negative control women agreed to take part in the study. None of the control women developed clinical or biochemical features associated with thyroid dysfunction during the postpartum period. Seventy-five of the antibody-positive women remained euthyroid during the postpartum year, while 73 showed biochemical evidence of thyroid dysfunction (PPT); a further four cases were excluded from the study because of pre-existing thyroid disease.

Laboratory studies
Blood samples were collected at booking, delivery and monthly for 12 months’ postpartum and the serum stored in aliquots at −20°C.

Free thyroid hormone (FT₄) and free triiodothyronine (FT₃) were measured using the Amerlex M methods (Amersham International, Chalfont, Bucks, UK) and thyrotrophin (TSH) by an enhanced luminescence immunoassay using the Amerlite TSH (monoclonal) reagents (Amersham International). The functional sensitivity of this assay was 0.04 mU/l (16). The reference ranges for thyroid function tests used in this study (FT₄ 4.2–7.7 pmol/l; FT₃ 8–19 pmol/l; TSH 0.5–3.6 mU/l) are based on the analysis of serum samples from 239 antibody-negative subjects included in the trial. All data points were analysed by ANOVA based on a quadratic regression (TSH values were not normally distributed and were analysed following log transformation). The ranges represent the 95% confidence intervals about the mean for FT₃ and FT₄ and the 95% confidence interval about the geometric mean in the case of TSH (17). An episode of thyroid dysfunction was defined as follows:

(i) Hyperthyroidism: either suppressed TSH together with FT₄ > 19 pmol/l or FT₃ > 7.7 pmol/l, or elevated FT₃ and FT₄ with either set of criteria occurring on one or more occasions.

(ii) Hypothyroidism: either TSH > 3.6 mU/l together with FT₄ < 8 pmol/l or FT₃ < 4.2 pmol/l, or TSH > 10 mU/l on one or more occasions.

Thyroid autoantibodies were determined by ELISA (18). Microtitre plates were coated overnight with sodium deoxycholate-solubilized Graves’ thyroid microsomal protein (Graves’ thyroid microsomes were prepared from snap-frozen tissue by differential centrifugation (18)). After washing, the coated wells were filled with 1:100 dilutions of patient serum (or standard dilutions) and incubated at room temperature for 2 h. This was followed by dilute peroxidase-conjugated sheep anti-human IgG. The bound peroxidase activity, seen as a green colour following the addition of ABST (Sigma Chemical Co., Poole, UK) and hydrogen peroxide as substrates, was measured at 550 nm in a Flow Multiscan spectrophotometer.

Thyrotrophin receptor antibodies were measured using porcine thyroid membranes and [¹²⁵I]bTSH (19).

Complement component C3b, immobilized as a result of classical complement pathway activation by TPO/TPO-ab complexes, was assayed in an ELISA using a peroxidase-conjugated goat anti-complement C3b antisem (20). Microtitre plates were coated with a human thyroid microsomal membrane fraction (prepared as described above) diluted to a protein concentration of 10 µg/ml in 50 mmol/l sodium carbonate (pH 9.1), incubated overnight at 4°C and washed. Replicate wells were set up containing diluent alone (negative control). NIBSC 66/387 reference preparation (primary standard)†, “high C3” secondary standard (serum obtained from an AIT patient and calibrated against NIBSC 66/387) or patient’s serum (100 µl/well of 1:100 dilution) and the plates incubated at room temperature for 2 h. Fresh guinea-pig serum as a source of complement (obtained from Gibco Ltd., Uxbridge, UK: cat. no. 065-9202 and stored frozen in aliquots at −70°C), diluted 1:200 in assay buffer, was added to the wells and the plates incubated at room temperature. After 1 h the plates were again washed and treated with peroxidase-conjugated goat anti-guinea pig C3 antisem (Cappel Ltd. no. 3207-0601, obtained from Dynatech Laboratories Ltd., Billingshurst, UK) and the degree of complement C3 activation was quantified following the addition of o-phenylenediamine (Sigma Chemical Co., Poole, UK) and hydrogen peroxide as substrates in 50 mmol/l citrate–phosphate buffer (pH 5.1). The reaction was stopped after 3 min at room temperature by the addition of 50 µl of 2 mol/l sulphuric acid and the resulting colour was measured in a Flow Multiscan spectrophotometer at 491 nm. A C3 index was calculated from the optical density (OD) of the standard

†NIBSC 66/387, the “Anti-thyroid microsome serum”, was obtained from the National Institute for Biological Standards and Control, Holly Hill, Hamstead, London NW3 6BB. UK and, with their approval, was assigned a complement C3 index of 1.00 for this assay.
Table 1. Thyroid peroxidase autoantibody (TPO-ab) levels in serum from different groups of women with postpartum thyroiditis (PPT) at delivery and 12 months postpartum, together with the peak level attained during the postpartum year (5–6 months’ postpartum). a

<table>
<thead>
<tr>
<th>Number of patients studied</th>
<th>TPO-ab level (kIU/l) (95% confidence interval)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Delivery</td>
</tr>
<tr>
<td>Euthyroid ab+</td>
<td>75</td>
</tr>
<tr>
<td>Hyperthyroid PPT only</td>
<td>14</td>
</tr>
<tr>
<td>Biphasic PPT</td>
<td>23</td>
</tr>
<tr>
<td>Hypothyroid PPT only</td>
<td>36</td>
</tr>
<tr>
<td>All Hypothyroid PPT</td>
<td>59</td>
</tr>
<tr>
<td>Transient PPT</td>
<td>44</td>
</tr>
<tr>
<td>Persistent PPT</td>
<td>29</td>
</tr>
</tbody>
</table>

aThe figures represent the geometric mean together with its 95% confidence interval and the time for which peak activity was recorded. Students t-test was used to compare groups of patients with the euthyroid antibody-positive group (p < 0.05 and **p < 0.005) and with the Hypothyroid-only PPT group (tp < 0.05 and †p < 0.0005) and to compare the transient and persistent PPT groups (†p < 0.05 and ‡p < 0.005). The reference range for this assay is <19.4 kIU/l.

and test samples using the calculation:

\[
\log_{10} \left( \frac{10^{\text{TEST OD} - \text{DILUENT OD}} - 1}{10^{\text{STANDARD OD} - 1}} \right) \times 10^{\text{STANDARD INDEX}}
\]

The bioactive TPO-ab activity was calculated as the product of the TPO-ab level and complement C3 index. It represents that part of the total TPO-ab able to interact with the complement system.

Data were analysed using the "SPSS" statistics package (SPSS Inc., Chicago, IL). Student's t-test was used to compare the groups. Data from the C3 index ELISA did not require transformation and are expressed as the mean value together with 95% confidence intervals. However, data for TPO-ab and bioactive TPO-ab activities were not normally distributed and required log transformation to achieve data skew values 0 ± 1. Following log transformation all antibody activities were expressed as geometric mean values with 95% confidence intervals.

Results

Based on the above definition of PPT, 73 women showed one or more episodes of thyroid dysfunction during the postpartum year. Of these women, 14 became hyperthyroid (median time of onset 3 months' postpartum; range 1–9 months' postpartum), 23 showed a biphasic PPT with sequential episodes of hyperthyroidism (median time of onset 2 months' postpartum; range 1–5 months) and hypothyroidism (median time of onset 4 months' postpartum; range 3–8 months). The remaining 36 women showed hypothyroid PPT only (median time of onset 4 months' postpartum, range 2–8 months). A further 75 TPO-ab-positive women remained euthyroid throughout the study period.

Euthyroid TPO-ab-positive group

The TPO-ab levels in the euthyroid TPO-ab-positive group of women (Table 1) were within the reference range for this assay (<19.4 kIU/l) at delivery. The antibody level rose steadily throughout the postpartum year to reach 121 kIU/l by 12 months' postpartum. This progressive increase in TPO-ab level was reflected in the values obtained for the complement C3 activation index (Table 2) and the calculated bioactive TPO-ab (Table 3).

Table 2. Data for the thyroid peroxidase (TPO)/TPO autoantibody complement C3 index obtained from different groups of women with postpartum thyroiditis (PPT) at delivery and 12 months' postpartum, together with the peak index obtained during the postpartum year (5–8 months’ postpartum). a

<table>
<thead>
<tr>
<th>Number of patients studied</th>
<th>Complement C3 index (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delivery</td>
</tr>
<tr>
<td>Euthyroid ab+</td>
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<tr>
<td>Persistent PPT</td>
<td>29</td>
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</tbody>
</table>

aThe figures represent the mean index together with its 95% confidence interval and the time at which the peak was recorded. Students t-test was used to compare groups of patients with the euthyroid antibody-positive group (p < 0.05 and **p < 0.005) and with the hyperthyroid-only PPT group, and to compare the transient and persistent PPT groups (†p < 0.05).
Table 3. The bioactive thyroid peroxidase autoantibody (TPO-ab) activity in the serum from different groups of women with postpartum thyroiditis (PPT) at delivery and 12 months' postpartum, together with the peak activity recorded during the postpartum year (6–9 months' postpartum).*

<table>
<thead>
<tr>
<th>Number of patients studied</th>
<th>Bioactive TPO-ab activity (95% confidence interval) (kIU/l)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Delivery</td>
</tr>
<tr>
<td>Euthyroid ab+</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0.4 (0.2–0.9)</td>
</tr>
<tr>
<td>Hyperthyroid PPT only</td>
<td>14</td>
</tr>
<tr>
<td>Biphagic PPT</td>
<td>23</td>
</tr>
<tr>
<td>Hypothyroid PPT only</td>
<td>36</td>
</tr>
<tr>
<td>All hypothyroid PPT</td>
<td>59</td>
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*The figures represent the geometric mean together with its 95% confidence interval and the time at which peak activity was recorded. Students t-test was used to compare groups of patients with the euthyroid antibody-positive group (p < 0.05 and **p < 0.005) and with the hypothyroid-only PPT group (†p < 0.05 and ††p < 0.005) and to compare the transient and persistent PPT groups (‡p < 0.05 and ‡‡p < 0.005).

Hyperthyroid PPT group

In 14 women who showed hyperthyroid PPT the TPO-ab level, C3 index and bioactive TPO-ab activity were lower than those seen in the euthyroid TPO-ab-positive group at delivery. The pattern of activity for each of these parameters in both these groups of women was statistically indistinguishable throughout the remainder of the postpartum year (Tables 1–3).

Biphagic and hypothyroid PPT groups

The 23 women who showed a biphagic PPT had low values for TPO-ab, C3 index and bioactive TPO-ab at delivery whilst the values obtained in the 36 women with hypothyroid PPT were slightly, though not significantly, higher (Tables 1–3). Thereafter, both groups showed progressively increasing values for each parameter, which peaked at about 6 months' postpartum. At this time all three parameters were elevated significantly (TPO-ab, p < 0.05; C3 index and bioactive TPO-ab, p < 0.005) compared with the euthyroid TPO-ab-positive group. Combining the data for the biphagic and hypothyroid PPT groups, which were statistically indistinguishable throughout the postpartum year, shows that not only were TPO-ab, C3 index and bioactive TPO-ab elevated compared to the euthyroid antibody-positive group (TPO-ab, C3 index and bioactive TPO-ab, p < 0.05) but during the time that the disease process was most active (0–6 months postpartum) all three parameters were raised significantly when compared with the hypothyroid PPT group (p < 0.05).

Transient and persistent thyroid dysfunction postpartum

An episode of hyperthyroidism during the postpartum year was always transient and usually resolved with a month of onset. However, some of the women who developed hypothyroidism failed to resolve during the course of the study. Women were considered to have a persistent hypothyroidism postpartum if, at the end of the postpartum year, their thyroid function tests remained outside the assay reference ranges or if they still required thyroxine replacement therapy (29 of 73 cases of PPT).

At delivery the values for TPO-ab, complement C3 index and bioactive TPO-ab in the transient PPT group were similar to those seen in the euthyroid TPO-ab-positive group (Tables 1–3). The TPO-ab levels remained broadly similar in these two groups throughout the remainder of the postpartum year, except that the level was raised significantly in the transient PPT group at 5 months' postpartum (p < 0.05). Values for the C3 index and bioactive TPO-ab activity were consistently higher in the transient PPT group than those seen in the euthyroid TPO-ab-positive group. C3 activation being significantly elevated at 4, 6 and 7 months' postpartum (p < 0.005) and bioactive TPO antibody activity being elevated significantly at all time points between 3 and 9 months' postpartum (p < 0.05).

In the persistent PPT group all three parameters were elevated significantly compared with the euthyroid TPO-ab-positive group at all time points (p < 0.005) and when compared with the transient PPT group (p < 0.05).

Thyrotrophin receptor antibody

Thyrotrophin receptor antibody activity was not detected in the serum of any of the women who experienced a hyperthyroid phase of PPT (data not shown).
Discussion

The manifestations of this Hashimoto-like autoimmune syndrome during the postpartum year range from the presence in the circulation of one or more autoantibodies directed against thyroid antigens, but without evidence of thyroid dysfunction, through transient episodes of hyperthyroidism and/or hypothyroidism, to a complete breakdown in thyroid function leading to persistent, and sometimes permanent, thyroid dysfunction.

The presence of a hyperthyroid phase in some cases of PPT (none of these women had postpartum Graves' disease (21)) is an unusual clinical presentation of classical Hashimoto's disease. This could be the result of a low level of a thyro-destructive mechanism, possibly complement mediated, resulting in the leakage of iodoproteins into the circulation to cause hyperthyroidism (22). The released hormone would, however, be in the form of a macromolecular complex that would require enzymatic cleavage to release the active form of the hormone into the circulation, a process normally catalysed by TPO (23). This sort of mechanism could explain the elevated serum thyroglobulin concentration (4) and high iodine excretion (3) seen at this time. However, an alternative explanation would be that this phenomenon is the result of a sublethal complement attack on the thyroid follicle.

Antibody-directed complement attack on nucleated cells can result in two quite distinct pathophysiological mechanisms (24). Under the influence of a sublethal complement attack the metabolic activity of cells is increased via a number of mechanisms, including a rapid rise in both intracellular Ca\(^{2+}\) ions (25) and cAMP (26). In the thyroid cell these metabolic changes would mimic the action of TSH (27), leading to the up-regulation of TPO (28) and secretion of thyroid hormones into the circulation, with consequent hyperthyroidism. However, in this event one might expect to see an increase in radioiodine uptake, and event that is not seen in PPT (29).

Should the complement-mediated attack be more severe, destruction of thyroid follicular architecture would ensue with a resulting loss of thyroid function, leading eventually to hypothyroidism. As the degree of complement activation increased, the transient hyperthyroid phase of the PPT could be missing altogether, the resulting PPT being characterized by hypothyroidism alone. In the most severe cases, damage to thyroid follicular architecture could be extensive and terminal and would result in a persistent Hashimoto-like hypothyroidism.

This scenario is reflected in the data described in this paper. Complement C3 activation indices and biochemical TPO-ab were marginally higher in the hyperthyroid PPT women compared to the euthyroid TPO-ab-positive women, although these changes did not achieve statistical significance. However, these parameters were lower than those seen in the women who proceeded to show biochemical evidence of hypothyroidism. Most striking were the values obtained for these parameters in women whose hypothyroidism failed to resolve before the end of the study period.

In the analysis of these data, artificial constraints have been imposed by the process of categorizing these women. The spectrum of the pathology of PPT from the presence of autoantibodies in euthyroid women through to a permanent thyroid dysfunction is continuous, with a number of factors combining to affect the outcome. It is clear from this study, however, that the activation of the complement cascade by thyroid autoantibodies could be an important factor in determining the progression of the thyroiditis.

There are distinct similarities between these data and those reported in cases of Hashimoto's thyroiditis (20), where the 95% confidence interval for the C3 activation index was 0.6–0.8 and for bioactive TPO-ab activity was 30–108 kIU/L. Whilst the mean values for these parameters in women with transient PPT failed to reach the levels of those in Hashimoto's thyroiditis, the values obtained with samples from women with persistent PPT are in excess of the lower limit of these ranges by 2 months' postpartum and thereafter remain elevated for the remainder of the study.

In conclusion, this study shows that the degree of thyroid pathology in women with PPT is related to the ability of circulating antithyroid autoantibodies to interact with and activate the complement cascade. Further, the persistent hypothyroidism seen in 40% of the PPT women examined in this study suggests that they have a Hashimoto-like condition.

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